1,2,4-Triazole Derivatives Inhibiting the Human Immunodeficiency Virus Type 1 (HIV-1) *in vitro*

by Irene M. Lagoja[a])[1]), Christophe Pannecouque*[b])[1]), Laura Musumeci[a]), Matheus Froeyen[a]), Arthur Van Aerschot[a]), Jan Balzarini[b]), Piet Herdewijn[a]), and Erik De Clercq[b])

[a]) Laboratory of Medicinal Chemistry
[b]) Laboratory of Virology and Chemotherapy, Rega Institute for Medical Research, Katholieke Universiteit Leuven, Minderbroedersstraat 10, B-3000 Leuven

Dedicated to Professor *Wolfgang Pfleiderer* on the occasion of his 75th birthday

A novel series of selective 1,2,4-triazole nonnucleoside reverse transcriptase inhibitors (NNRTIs) is described. In MT-4 cells compound, **4f** inhibited human immunodeficiency virus type 1 (HIV-1) induced cytopathology at an IC_{50} of 9.98 μM with a selectivity index of 18.6. The hypothetical docking model of RT/**4f** derived from X-ray crystallographic structure of capravirine complex with HIV-1 RT links the activity profile to the H-bonding network.

1. Introduction. – Replication of human immunodeficiency virus type 1 (HIV-1) can be reduced in HIV-1-infected patients with a combination of antiviral drugs targeted at the reverse transcriptase (RT) and protease (PR) [1]. Unfortunately, due to the high mutation rate of HIV, treatment, even drug combinations, select for drug-resistant HIV variants [2]. There is a continuous need for new drugs that are active against clinical drug-resistant mutant strains and/or aimed at new targets in the viral replicative cycle [3].

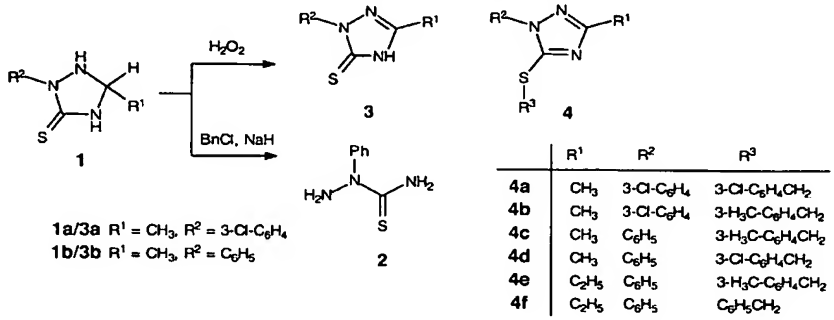
The HIV RT is necessary for the conversion of the HIV single-stranded RNA to double-stranded DNA [4]. Several clinically used drugs do have RT as a target. These drugs either compete with the natural 2′-deoxynucleoside-5′-*O*-triphosphates or are targeted at an allosteric, nonnucleoside binding site [5]. The latter compounds are referred to as NNRTIs (nonnucleoside reverse transcriptase inhibitors). They constitute a group of chemically dissimilar molecules that bind to a hydrophobic pocket near, but distinct from, the polymerase active site of the p66 subunit. The enzymatic activity of RT is inhibited by allosteric changes in the enzyme that cause a distortion of the catalytic active-site aspartyl residues [6]. To the NNRTIs belongs capravirine (S-1153), a 1,2,4,5-substituted imidazole derivative [7], which is able to inhibit HIV-1 strains that are resistant to other NNRTIs. This ability to cope with typical NNRTI-induced mutations is based, at least in part, on an extensive network of H-bonds involving the main chain of residues 101, 103, and 236 of the p66 RT subunit [8]. With the structure of capravirine as a lead compound, we synthesized a series of 1,2,4-triazole derivatives and determined their anti-HIV activity in a cell-based assay [9].

[1]) Both authors contributed equally.

The 3-ethyl-1-phenyl-5-(benzylsulfanyl)-1*H*-[1,2,4]triazole **4f** had low cytotoxicity (50% cytotoxic concentration (CC_{50}: 185.20 μM)) and showed anti-HIV-1 activity at a 50% inhibitory concentration (IC_{50}) of 9.98 μM (selectivity index 18.6). The selective activity of this and related compounds, **4c** and **4d**, against HIV-1(III_B) and their inactivity against an NNRTI-resistant (RT K103N and Y181C) mutant, HIV-2(ROD), and simian immunodeficiency virus (SIV(mac251)) clearly indicate that these compounds qualify as NNRTIs and are targeted at HIV-1 RT.

2. Chemical Results and Discussion. – Using capravirine as our lead substance, we synthesized a new group of five-membered ring heterocycles, carrying two bulky substituents in *ortho* to each other. The 1,2,4-triazole ring was selected as the heterocyclic scaffold for the substituents because its chemistry is well-known. Indeed, diverse biological applications have been reported for 5-alkyl-2-aryl-1,2,4-triazolidine-3-thiones **1** [10] (*Scheme 1*), which possess a similar arrangement of substituents.

Scheme 1. *Synthesis of 5-Alkyl-2-aryl-1,2,4-triazolidine-3-thiones* **1**, *3-Alkyl-1-aryl-4,5-dihydro-1*H*-1,2,4-triazole-5-thiones* **3**, *and 3-Alkyl-1-aryl-5-[(arylmethyl)sulfanyl]-1*H*-1,2,4-triazoles* **4**



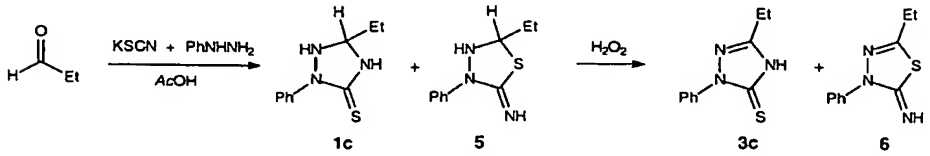
	R^1	R^2	R^3
4a	CH_3	3-Cl-C_6H_4	3-Cl-$C_6H_4CH_2$
4b	CH_3	3-Cl-C_6H_4	3-H_3C-$C_6H_4CH_2$
4c	CH_3	C_6H_5	3-H_3C-$C_6H_4CH_2$
4d	CH_3	C_6H_5	3-Cl-$C_6H_4CH_2$
4e	C_2H_5	C_6H_5	3-H_3C-$C_6H_4CH_2$
4f	C_2H_5	C_6H_5	$C_6H_5CH_2$

In a one-pot reaction, 5-alkyl-2-aryl-1,2,4-triazolidine-3-thiones **1** can be obtained in good yield from aldehydes, hydrazines, and KSCN [11]. To introduce a second bulky substituent, a benzylation reaction was attempted. However, direct benzylation with BnCl as a *Mitsunobu* reaction [12] yielded *N*-amino-*N*-phenylthiourea (**2**). Therefore, the 1,2,4-triazolidine-3-thiones **1** were first oxidized with H_2O_2 to obtain the more-stable aromatic 3-alkyl-1-aryl-4,5-dihydro-1*H*-1,2,4-triazole-5-thiones **3**, which could subsequently easily be benzylated to the corresponding 3-alkyl-1-aryl-5-[(arylmethyl)-sulfanyl]-1*H*-1,2,4-triazoles **4** (*Scheme 1*).

However, with propanal as the starting material to obtain the 5-Et analogues, the reaction was not straightforward. Reaction of propanal with phenylhydrazine and KSCN did not lead to a single product. An unseparable 1:1 mixture of two compounds with the same molecular weight was obtained. Likewise, following the oxidation step, no separation was possible. In the past [11], a structure with an endocyclic S-atom has been considered a possible product of the ring closure of hydrazones and KSCN, but was later ruled out for chemical reasons. Here, a 1:1 mixture of 5-ethyl-2-phenyl-1,2,4-triazolidine-3-thione (**1c**) and 5-ethyl-3-phenyl-1,3,4-thiadiazolidin-2-imine (**5**) was

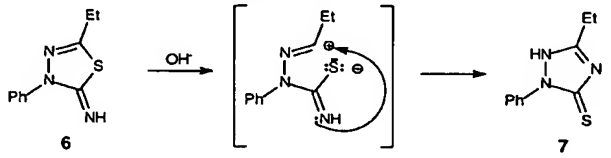
obtained and oxidized to the corresponding mixture of 3-ethyl-4,5-dihydro-1-phenyl-1*H*-1,2,4-triazole-5-thione (**3c**) and 5-ethyl-3-phenyl-1,3,4-thiadiazol-2(3*H*)-imine (**6**) (*Scheme 2*).

Scheme 2. *Synthesis of the 1,2,4-Triazolidine-3-thione and 1,3,4-Thiadiazolidine-2-imine Derivatives* **1c** *and* **5**, *Respectively, and Their Oxidation Products* **3c** *and* **6**, *Respectively.*

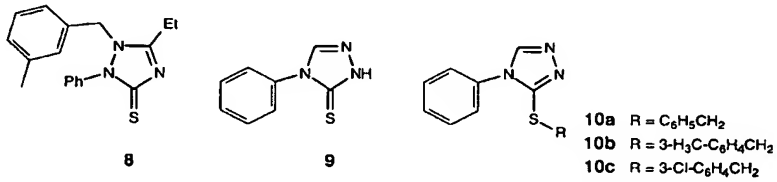


A triazole derivative could be obtained by a further rearrangement of **6**. Indeed, through a *Dimroth* rearrangement [13], **6** could be transformed into 5-ethyl-2,3-dihydro-2-phenyl-1*H*-1,2,4-triazole-3-thione (**7**) (*Scheme 3*).

Scheme 3. Dimroth *Rearrangement of 1,3,4-Thiadiazol-2(3*H*)-imine* **6**.



The two isomers **3c** and **7** could be separated by column chromatography. The position of the NH was determined by way of NOE-difference spectroscopy. Irradiation of the NH gave rise to a positive NOE enhancement of the Et as well as the Ph moiety for **7**, whereas, in case of **3c**, only a positive NOE enhancement for the Et group could be observed. Benzylation of **3c** yielded the corresponding 3-ethyl-1-phenyl-5-[(phenylmethyl)sulfanyl]-1*H*-1,2,4-triazole (**4c**), whereas, in the case of **7** under the same reaction conditions (NaH, benzyl chloride), the corresponding 5-ethyl-2,3-dihydro-1-[(2-methylphenyl)methyl]-2-phenyl-1*H*-1,2,4-triazole-3-thione (**8**) could be obtained.



We also decided to synthesize an example of a 1,2,4-triazole derivative with a different substitution pattern on the heterocyclic ring. The Ph ring remained in α-position with respect to the benzylsulfanyl substituent, but, in contrast to the previous series, it was substituted at N(3) instead of N(2). This alters the ring geometry. The isomeric 4,5-dihydro-4-phenyl-1*H*-1,2,4-triazole-5-thione (**9**) [14] were obtained by

cyclization of 4-(phenylsulfanyl)semicarbazide with HCOOEt. Subsequent benzylation yielded 3-[(arylmethyl)sulfanyl]-4-phenyl-4*H*-1,2,4-triazoles **10**.

3. Biological Results and Discussion. – We evaluated a series of substituted 1,2,4-triazole derivatives for their potential to inhibit the *in vitro* replication of HIV and SIV in a cell-culture model for acute infection. The antiviral activity and cytotoxicity data are presented in *Table 1*.

Table 1. *Inhibition of HIV-1, HIV-2, and SIV Replication in MT-4 Cells by Triazole Derivatives*

Compound	IC_{50} [μM][a])				CC_{50} [μM][b])
	HIV-1		HIV-2 ROD	SIV mac251	
	III_B	NNRTI[c])			
1a	> 42.02	ND	> 42.02	> 42.02	42.02 ± 19.19
3c	> 243.58	ND	> 243.58	> 243.58	> 243.58
4a	> 60.70	ND	> 60.70	> 60.70	60.70 ± 15.33
4b	> 31.41	ND	> 31.41	> 31.41	31.41 ± 15.88
4c	17.30 ± 0.85	> 72.65	> 72.65	> 72.65	72.65 ± 14.82
4d	16.88 ± 4.94	> 76.24	> 76.24	> 76.24	76.24 ± 19.88
4e	> 40.62 (25–37%)[d])	> 58.50	> 58.50	> 58.50	58.50 ± 14.38
4f	9.98 ± 4.37	> 185.20	> 185.20	> 185.20	185.20 ± 80.57
7	> 243.6	ND	> 243.6	> 243.6	> 243.6
8	46.63 ± 21.33	> 118.64	> 118.64	> 118.64	118.61 ± 3.20
10a	> 201.54	ND	> 201.54	> 201.54	201.54 ± 36.99
10b	> 152.15(15–42%)[d])	> 152.15	> 152.15	> 152.15	152.15 ± 51.28
10c	> 87.41	ND	> 87.41	> 87.41	87.41 ± 10.20

[a]) 50% Inhibitory concentration, or concentration required to inhibit the viral cytopathic effect by 50% in MT-4 cells. Data represent average values ± SD for at least two independent experiments. [b]) 50% Cytotoxic concentration, or concentration that reduced MT-4 cell viability by 50%. [c]) NNRTI-Resistant HIV-1 strain. [d]) Percent inhibition of virus replication at the indicated concentration is mentioned between parentheses.

Anti-HIV activity was observed for compounds **4c**, **4d**, **4f**, and **8**, of which the former three compounds, **4c**, **4d**, and **4f**, are structurally related. The basis of these three molecules is 3-alkyl-5-(benzylsulfanyl)-1-phenyl-1*H*-1,2,4-triazole. From comparison of the *in vitro* antiretroviral activity of this subgroup of molecules, it appears that a *m*-Cl substituent on the 5-phenylsulfanyl moiety is deleterious for the antiretroviral activity (**4a** *vs.* **4d**, and **4b** *vs.* **4c**). *m*-Me *vs.* a *m*-Cl substitution (compounds **4c** and **4d**, resp.) gave similar anti-HIV activity and cytotoxicity. Comparison of the 3-Me *vs.* the 5-Et derivative (**4c** *vs.* **4e**) revealed that the 3-Me compound was slightly more active and slightly less cytotoxic. Comparison of **4f** and **4e** indicated that 3-methylation of the benzylsulfanyl group rendered the compound less active. Comparing **3c** with its benzylated derivative **4f** clearly established the necessity of the benzyl moiety for anti-HIV activity. Displacement of the 1-aryl moiety to the 4-position gave the triazole derivatives inactive (**4c** and **4d** *vs.* **10c** and **10b**).

When the 1,2,4-triazole derivatives were evaluated for their inhibitory activity against HIV-1 RT, compound **4f** was inhibitory at an IC_{50} of 156 and 128 μM in the presence of poly(rA·dT) and poly(rC·dG), respectively, as the template/primer (*Table 2*). Compounds **4e**, **10b**, and **8** inhibited the reaction by 50% at concentrations of

Table 2. *Inhibitory Activity of Triazole Derivatives against Recombinant HIV-1 RT with Poly (rC·dG) or Poly (rA·dT) as the Template/Primer, and [^{3}H]dGTP and [^{3}H]dTTP as the Radiolabeled Substrate*

Test compounds	IC_{50} [μM][a])	
	Poly (rA·dT) (dTTP)	Poly (rC·dG) (dGTP)
4c	> 710 (8%)[b])	> 710 (24%)
4d	> 633 (21%)	> 633 (39%)
4e	> 646 (37%)	> 646 (45%)
4f	156	128
8	> 646 (45%)	594
10b	710	632

[a]) 50% Inhibitory concentration or compound concentration required to inhibit HIV-1 RT activity by 50%. [b]) Percent enzyme inhibition at the indicated concentration is mentioned between parentheses.

ca. 600–700 μM. Thus, the most efficient inhibitor of HIV-1, *i.e.*, **4f**, was also most inhibitory to HIV-1 RT. Compound **4c** afforded slight inhibition of the RT reaction at the highest concentration tested (*ca.* 710 μM).

4. Computer-Aided Ligand Design. – The crystal structure of HIV-1 RT with capravirine (pdb entry 1EP4) was used as starting point [8]. All H_2O molecules were removed as well as the S-1153 inhibitor. The structure **4f** was drawn in Macromodel and optimized in the AM1 force field [15]. We used a flexible superposition program that can vary the dihedral angles of molecule **4f**, superimposing this molecule onto the reference molecule capravirine with the SEAL program [16] and optimizing the resulting score with an adaptive simulated annealing optimizer [17] (*Fig. 1,a*). The final structure was then put in the NNRTI site of RT. The topology and amber force field parameters for **4f** were constructed based on existing similar parameter values in the amber5 software [18]. Electrostatic charges were calculated by means of the GAMESS [19] and RESP methods [20].

The complex was then energy-minimized, and the results were examined with RasMol [21] (*Fig. 1,b*). We assume that molecules **10x** bind in the same way as molecules **4x**, as the ring systems of both molecule series can be superimposed completely. Following superposition, we obtain an apparent swap of N(2) with C(3) of the triazole ring.

The capravirine-RT complex is stabilized by two H-bonds from the carbamate group of capravirine to P236 main-chain O-atom and K103 backbone N-atom. As our molecules **4x** and **10x** lack a carbamate group, which could contribute to the binding of the new inhibitors, a lower affinity is expected compared to capravirine (*Fig. 2*). A third H-bond is observed from the five-ring N-atom N(2) to K101 main-chain CO, mediated by a H_2O molecule (distance N(2)···K101.N 4.09 Å). In our RT-**4f** model, N(2) of the triazole ring may possibly make an analogous H_2O-mediated H-bond with K101 backbone O-atom (distance **4f** N(2)···K101.O 4.64 Å). However, this interaction with N(2) is also possible for the **10x** series with N(1) taking the position of C(3) (distance **4f** C(3)···K101.O 4.47 Å). An additional H_2O-mediated H-bond might be formed from N(1) to the Y318 OH group. Based on the distances (**4f** N(1)···Y318.OH

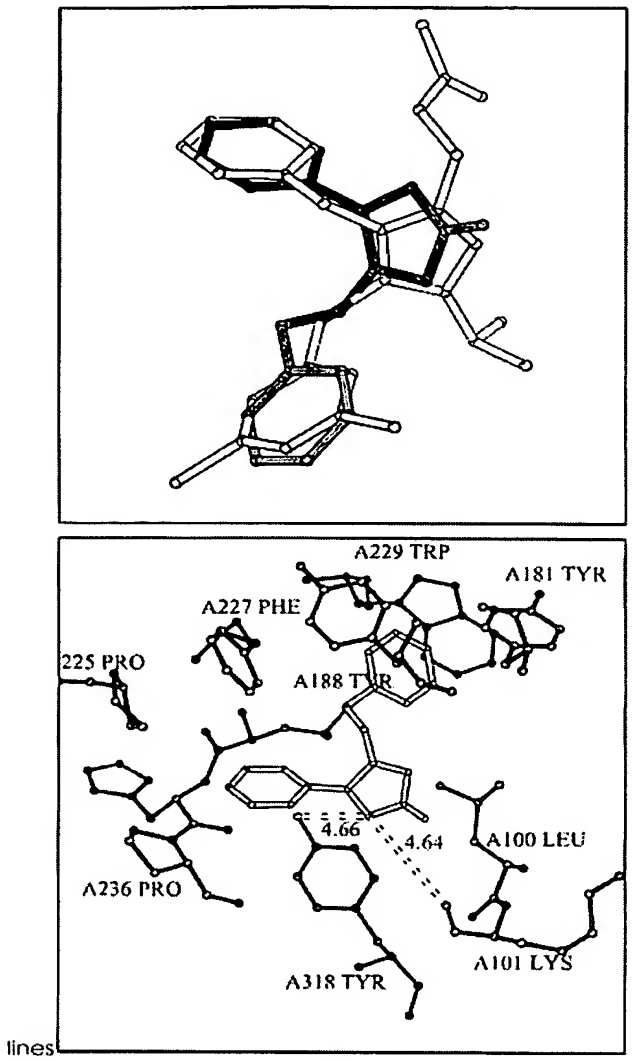


Fig. 1. a) *Flexible-seal fit of compound* **4f** *onto capravirine*, b) *model of compound* **4f7** *bound to the NNRTI site of HIV-1 RT*. The distances from the atom N(2) to K101.O and Y318.OH are indicated with dashed lines.

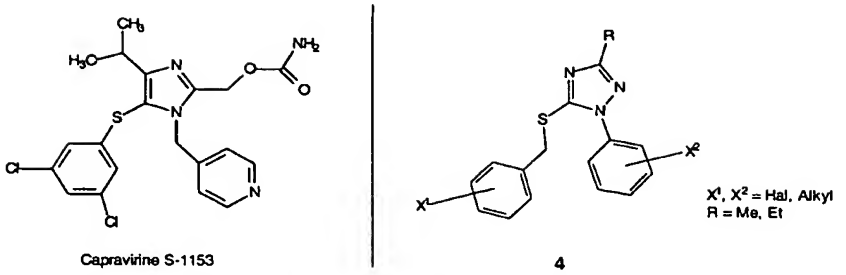


Fig. 2. *Structure analogy of capravirine* vs. *triazole derivatives* **4**

4.66 Å, **4f** C(3)···Y318.OH 5.38 Å), this is more likely the case for the **4x** series than for the **10x** series of molecules, which could partially explain the differences in binding strength and activity.

5. Conclusions. – We synthesized a series of [1,2,4] triazole derivatives based on the structure of capravirine. Some of these compounds inhibited the HIV replication *in vitro*, based on an NNRTI type of action.

Experimental Part

General. For all reactions, anal. grade solvents were used. Compound **9** was prepared according to the procedure described in [14]. TLC was performed on TLC aluminium sheets (*Merck*, silica gel *60* F_{254}) and silica (200–425 mesh) was used for column chromatography (CC). M.ps. were determined with a *Büchi SMP-20* cap. melting-point apparatus. NMR Spectra were recorded on a *Varian Gemini-200* spectrometer (^{1}H: 200 MHz, ^{13}C: 50 MHz; ^{13}C and ^{1}H signals refer to TMS; all NH/OH protons were assigned by exchange with D_2O. Exact mass measurements were performed on a quadrupole time-of-flight mass spectrometer (*Q-Tof-2*, *Micromass*, Manchester, UK) equipped with a standard electrospray-ionization (ESI) interface; samples were infused in i-PrOH/H_2O 1:1 at 3 µl/min.

Synthesis 5-Alkyl-2-aryl-1,2,4-triazolidine-3-thiones (**1**): *General Procedure.* To a stirred soln. of an aldehyde (2.5 mmol) in AcOH (10 ml), hydrazine (2.5 mmol) or hydrazine hydrochloride was added. After 30 min, KSCN (0.37 g, 3.8 mmol) was added at r.t. After stirring for 5 h at 30°, the mixture was checked with TLC (CH_2Cl_2/MeOH 99:1). After adding 50 ml of H_2O, the precipitate was filtered off and washed with H_2O. Mostly, recrystallization from MeOH afforded pure products.

2-(3-Chlorophenyl)-5-methyl-1,2,4-triazolidine-3-thione (**1a**). Yield 55.5%. M.p. 128–130° (MeOH). R_f (CH_2Cl_2/MeOH 9:1) 0.83. ^{1}H-NMR ((D$_6$)DMSO): 1.34 (*d*, *J* = 6, Me); 5.19 (*q*, *J* = 5.8, H–C(5)); 7.00 (*t*, *J* ≈ 8.8, H–C(4), H–C(6) of C_6H_4); 7.06 (*s*, H–C(2) of C_6H_4); 7.32 (*t*, *J* ≈ 8, H–C(5) of C_6H_4); 9.16 (*s*, NH); 10.58 (*s*, NH). ^{13}C-NMR ((D$_6$)DMSO): 22.4 (Me); 114.5, 115.4, 122.2, 130.9, 133.8, 152.5 (C(6), C(5), C(4), C(2), C(3), C(1) of C_6H_4); 78.6, 177.8 (C(5), 3-C=S). HR-MS: 228.0369 ([*M* + 1]$^+$ $C_6H_{10}ClN_3S$; calc. 228.0362).

5-Methyl-2-phenyl-1,2,4-triazolidine-3-thione (**1b**). Yield 61.4%. M.p. 169–171°. R_f (CH_2Cl_2/MeOH 9:1) 0.83. ^{1}H-NMR ((D$_6$)DMSO): 1.33 (*d*, *J* = 5.8, Me); 5.05 (*q*, *J* = 5.8, H–C(5)); 7.00 (*dd*, *J* = 5.4, 4.2, H–C(3), H–C(5) of Ph); 7.26–7.33 (H–C(2), H–C(4), H–C(6) of Ph); 7.03 (*s*, NH); 8.94 (*s*, NH); 10.31 (*s*, NH). ^{13}C-NMR ((D$_6$)DMSO): 21.4 (Me); 116.3, 123.1, 129.4, 151.4 (C(2), C(6); C(4); C(3), C(5); C(1) of Ph); 79.3, 177.7 (C(5), 3-C=S). HR-MS: 194.0748 ([*M* + 1]$^+$, $C_9H_{11}N_3S$; calc. 194.0752).

Oxidation Reaction: General Procedure. To a stirred soln. of **1** (100 mg, 0.52 mmol) in acetone (5 ml), H_2O_2 (*ca.* 0.1 mmol) was added. After stirring for 1 h at r.t., the mixture was checked by TLC (CH_2Cl_2/MeOH 99:1). After removing the solvent under reduced pressure, the product was either purified by CC or directly used for further reactions.

1-(3-Chlorophenyl)-4,5-dihydro-3-methyl-1H-1,2,4-triazole-5-thione (**3a**). Yield 80%. M.p. 144–146°. R_f (CH_2Cl_2/MeOH 99:1) 0.77. ^{1}H-NMR ((D$_6$)DMSO): 2.55 (*s*, Me); 7.34–7.48 (*m*, 4 arom. H); 8.42 (*s*, NH).

^{13}C-NMR ((D$_6$)DMSO): 13.2 (Me); 122.4, 124.7, 129.2, 130.4, 135.1, 137.7 (C(6), C(2), C(4), C(5), C(1), C(3) of C_6H_4); 154.3, 159.2 (C(5), C=S). HR-MS: 226.0238 ([$M+1$]$^+$, $C_9H_9ClN_3S^+$; calc. 226.0206).

4,5-Dihydro-3-methyl-1-phenyl-1H-1,2,4-triazole-5-thione (**3b**): Yield 97.5%. M.p. 105–107°. R_f (CH_2Cl_2/MeOH 99:1): 0.85. ^{1}H-NMR ((D$_6$)DMSO): 2.43 (*s*, Me); 7.35–7.37 (*m*, 5 arom. H); 8.35 (*s*, NH); ^{13}C-NMR ((D$_6$)DMSO): 13.0 (Me); 124.5, 129.3, 129.6, 136.8 (C(2), C(6); C(4); C(3), C(5); C(1) of Ph); 153.9, 158.8 (C(5), 3-C=S). HR-MS: 192.0598 ([$M+1$]$^+$, $C_9H_{10}N_3S$; calc. 192.0595).

3-Ethyl-4,5-dihydro-1-phenyl-1H-1,2,4-triazole-5-thione (**3c**): Yield 41.6%. M.p. 188–191°. R_f (AcOEt/hexane 2:1) 0.79. Exact mass ($C_{10}H_{11}N_3S$) [M + H]$^+$: Calc. 206.0752, found: 206.0758. ^{1}H-NMR ((D$_6$)DMSO): 1.22 (*t*, J = 7.4, Me); 2.63 (*q*, J = 7.4, CH_2); 7.55 (*d*, J = 6, H–C(2), H–C(6) of Ph); 7.24–7.38 (*m*, H–C(8), H–C(4), H–C(5) of Ph); 8.36 (*s*, NH). ^{13}C-NMR ((D$_6$)DMSO): 12.2 (Me); 21.5 (CH_2); 124.5, 129.2, 129.5, 136.8 (C(2), C(6); C(4); C(3), C(5); C(1) of Ph); 152.7, 158.8 (C(5), C=S). HR-MS: 206.0758 ([$M+1$]$^+$, $C_{10}H_{11}N_3S^+$; calc. 206.0752).

Benzylation Reaction: General Procedure. To a stirred soln. of **3** or **7** (1.55 mmol) in MeCN (10 ml), NaH (100 mg, 50% suspension in H_2O) and, after 1 h, the corresponding BnCl (1.55 mmol) was added. The mixture was left overnight at r.t., and then evaluated by TLC (CH_2Cl_2/MeOH 97:3). After removing the solvent, the product was purified by CC (CH_2Cl_2/MeOH 97:3).

1-(3-Chlorophenyl)-5-[[(3-chlorophenyl)methyl]sulfanyl]-3-methyl-1H-1,2,4-triazole (**4a**). Yield 62.6%. Oil. R_f (CH_2Cl_2/MeOH 99:1) 0.51. Exact mass ($C_{16}H_{14}N_3SCl_2$) [M + H]$^+$: Calc. 350.0285, found: 350.0302. ^{1}H-NMR ($CDCl_3$): 2.49 (*s*, Me); 4.31 (*s*, CH_2); 7.19–7.45 (*m*, 8 arom. H). ^{13}C-NMR ($CDCl_3$): 13.1 (Me); 35.2 (CH_2); 121.9, 124.4, 129.1, 130.3, 135.0, 138.0 (C(6), C(2), C(4), C(5), C(1), C(3) of ClC_6H_4), 127.1, 127.3, 128.6, 129.6, 133.9, 139.5 (C(6), C(2), C(5), C(4), C(3), C(1) of $ClC_6H_4CH_2$); 153.2 (C(3), 159.7 (C(5)). HR-MS: 350.0302 ([$M+1$]$^+$; $C_{16}H_{14}Cl_2N_3S^+$; calc. 350.0285).

1-(3-Chlorophenyl)-3-methyl-5-[[(3-methylphenyl)methyl]sulfanyl]-1H-1,2,4-triazole (**4b**). Yield 63.5%. Oil. R_f (CH_2Cl_2/MeOH 95:5) 0.83. ^{1}H-NMR ($CDCl_3$): 2.31 (*s*, MeC_6H_4); 2.47 (*s*, Me–C(3)); 4.34 (*s*, CH_2); 7.02–7.47 (*m*, 8 arom. H). ^{13}C-NMR ($CDCl_3$): 13.0 (*Me*–C(3)); 21.0 (*Me*–C_6H_4); 35.8 (CH_2); 121.8, 124.2, 129.1, 130.2, 134.9, 138.0 (C(6), C(2), C(4), C(5), C(1), C(3) of ClC_6H_4); 125.9, 127.9, 128.2, 128.4, 136.9, 137.8 (C(6), C(2), C(4), C(5), C(3), C(1) of MeC_6H_4); 152.9 (C(3), 160.3 (C(5)). HR-MS: 330.0832 ([$M+1$]$^+$, $C_{17}H_{17}ClN_3S^+$; calc. 330.0832).

3-Methyl-5-[[(3-methylphenyl)methyl]sulfanyl]-1-phenyl-1H-1,2,4-triazole (**4c**). Yield 60%. Oil. R_f (CH_2Cl_2/MeOH 99:1) 0.41. ^{1}H-NMR ($CDCl_3$): 2.32 (*s*, MeC_6H_4); 2.49 (Me–C(3)); 4.36 (*s*, CH_2); 7.19–7.26 (*m*, 5 H); 7.38–7.51 (*m*, 5 H). ^{13}C-NMR ($CDCl_3$): 13.0 (C(3)); 21.19 (MeC_6H_4); 36.0 (CH_2); 124.4, 129.4, 129.8, 137.0 (C(2), C(6); C(4); C(3), C(5); C(1) of Ph); 126.2, 128.1, 128.3, 129.8, 137.1, 137.3 (C(6), C(2), C(4), C(5), C(3), C(1) of MeC_6H_4); 153.1 (C(3)); 160.0 (C(5)). HR-MS: 296.1234 ([$M+1$]$^+$ $C_{17}H_{18}N_3S^+$; calc. 296.1221).

5-[[(3-Chlorophenyl)methyl]sulfanyl]-3-methyl-1-phenyl-1H-1,2,4-triazole (**4d**): Yield 66%. Oil; R_f (CH_2Cl_2/MeOH 97:3) 0.26. ^{1}H-NMR ($CDCl_3$): 2.47 (*s*, Me); 4.31 (*s*, CH_2); 7.19–7.49 (*m*, 9 H). ^{13}C-NMR ($CDCl_3$): 12.9 (Me), 35.3 (CH_2); 124.2, 127.3, 129.3, 139.7 (C(2), C(6); C(3), C(5); C(4); C(1) of Ph), 127.2, 128.6, 129.2, 129.6, 133.9, 139.7 (C(6), C(2), C(5), C(4), C(3), C(1) of ClC_6H_4); 153.2 (C(3)); 159.3 (C(5)). HR-MS: 316.0690 ([$M+1$]$^+$, $C_{16}H_{15}ClN_3S$; calc. 316.0675).

3-Ethyl-5-[[(3-methylphenyl)methyl]sulfanyl]-1-phenyl-1H-1,2,4-triazole (**4e**). Yield 65%. Oil. R_f (CH_2Cl_2/MeOH 99:1) 0.82. ^{1}H-NMR ($CDCl_3$): 1.38 (*t*, J = 6.6, Me); 2.31 (*s*, MeC_6H_4); 2.80 (*q*, J = 6.6, CH_2); 4.44 (*s*, 2 H); 7.08–7.27 (*m*, 4 H); 7.35–7.50 (*m*, 5 arom. H of Ph). ^{13}C-NMR ($CDCl_3$): 12.5 (Me); 21.2 (MeC_6H_4); 21.8 (CH_2), 37.9 ($C_6H_4CH_2$); 123.9, 129.2, 129.9, 136.1 (C(4); C(2), C(6); C(3), C(5), C(1) of Ph); 124.1, 124.9, 126.2, 128.3, 129.4, 129.9 (C(6), C(5), C(4), C(2), C(1), C(3) of MeC_6H_4); 152.7 (C(3)); 160.2 (C(5)). HR-MS: 310.1395 ([$M+1$]$^+$, $C_{18}H_{20}N_3S^+$; calc. 310.1378).

3-Ethyl-1-phenyl-5-[(phenylmethyl)sulfanyl]-1H-1,2,4-triazole (**4f**). Yield 60%. Oil. R_f (CH_2Cl_2/MeOH 99:1) 0.52. ^{1}H-NMR ($CDCl_3$): 1.30 (*t*, J = 7.6, Me); 2.77 (*q*, J = 7.6, CH_2); 4.40 (*s*, CH_2); 7.26–7.48 (*m*, 10 H). ^{13}C-NMR ($CDCl_3$): 12.4 (Me); 20.1 (CH_2); 36.24 ($PhCH_2$); 124.9, 129.5, 130.1, 137.5 (C(2), C(6); C(4); C(3), C(5); C(1) of Ph); 127.3, 128.5, 128.9, 129.2 (C(2), C(6); C(4); C(3), C(5); C(1) of $PhCH_2$); 152.9 (C(3); 160.0 (C(5)). HR-MS: 296.1237 ([$M+1$]$^+$, $C_{17}H_{18}N_3S^+$; calc. 296.1221).

5-Ethyl-2,3-dihydro-1-[(3-methylphenyl)methyl]-2-phenyl-1H-1,2,4-triazole-3-thione (**8**). Yield 43.5%. Oil. R_f (CH_2Cl_2/MeOH 99:1) 0.95. ^{1}H-NMR ($CDCl_3$): 1.35 (*t*, J = 7.6, Me); 2.17 (*s*, Me); 2.80 (*q*, J = 7.6, CH_2); 4.36 (*s*, CH_2); 7.09–7.51 (*m*, 9 H). ^{13}C-NMR ($CDCl_3$): 12.4 (Me); 20.1 (CH_2); 21.3 (MeC_6H_4); 30.8 ($C_6H_4CH_2$); 124.8, 126.5, 129.8, 142.5 (C(2), C(6); C(3), C(5); C(4); C(1) of Ph); 126.2, 128.3, 128.8, 129.2, 137.9, 138.0 (C(6), C(5), C(4), C(2), C(3), C(1) of MeC_6H_4); 158.4 (C(5)); 160.2 (C(3)). HR-MS: 310.1386 ([$M+1$]$^+$, $C_{18}H_{19}N_3S^+$; calc. 310.1378).

4-Phenyl-3-[(phenylmethyl)sulfanyl]-4H-1,2,4-triazole (**10a**). Yield 85.4%. M.p. 88–91°. R_f (CH_2Cl_2/MeOH 97:3) 0.72. ^{1}H-NMR ($CDCl_3$): 4.44 (*s*, CH_2); 7.17–7.30 (*m*, 7 arom. H); 7.42–7.44 (*m*, 3 arom. H); 8.26 (*s*, H–C(5)). ^{13}C-NMR ($CDCl_3$): 37.1 (CH_2); 124.8, 128.7, 129.4, 135.9 (C(2), C(6); C(3), C(5); C(4); C(1) of Ph); 127.4, 128.2, 128.8, 132.9 (C(4); C(2), C(6); C(3), C(5); C(1) of Bn); 144.1, 150.1 (C(5), C(3)). HR-MS: 268.0920 ($[M+1]^+$, $C_{15}H_{14}N_3S^+$; calc. 268.0908).

3-[[(3-Methylphenyl)methyl]sulfanyl]-4-phenyl-4H-1,2,4-triazole (**10b**). Yield 75.4%. Oil. R_f (CH_2Cl_2/MeOH 97:3) 0.92. ^{1}H-NMR ($CDCl_3$): 2.27 (*s*, Me); 4.43 (*s*, CH_2); 7.06–7.22 (*m*, 6 arom. H); 7.43–7.47 (*m*, 3 arom. H); 8.30 (*s*, H–C(5)). ^{13}C-NMR ($CDCl_3$): 20.9 (Me); 37.3 (CH_2); 124.9, 129.0, 129.6, 135.7 (C(2), C(6); C(3), C(5); C(4); C(1) of Ph)), 125.4, 128.0, 128.3, 128.8, 136.9, 138.1 (C(6), C(2), C(4), C(5), C(5), C(1) of MeC_6H_4); 144.2, 150.5 (C(5), C(3). HR-MS: 282.1062 ($[M+1]^+$, $C_{16}H_{16}N_3S^+$; calc. 282.1065).

3-[[(3-Chlorophenyl)methyl]sulfanyl]-4-phenyl-4H-1,2,4-triazole (**10c**). Yield 70%. Oil. R_f (CH_2Cl_2/MeOH 95:5) 0.81. ^{1}H-NMR ($CDCl_3$): 4.43 (*s*, CH_2); 7.19–7.32 (*m*, 6 arom. H); 7.46–7.50 (*m*, 3 arom. H); 8.30 (*s*, H–C(5)). ^{13}C-NMR ($CDCl_3$): 36.6 (CH_2); 125.1, 129.1, 129.6, 134.3 (C(2), C(6); C(3), C(5); C(4); C(1) of Ph); 127.3, 127.9, 129.5, 129.8, 133.1, 138.4 (C(6), C(5), C(4), C(2), C(1), C(3) of $C_6H_4CH_2$); 144.4, 150.1 (C(5), C(3)). HR-MS: 302.0511 ($[M+1]^+$, $C_{15}H_{13}ClN_3S^+$; calc. 302.0519).

Synthesis of 5-ethyl-2,3-dihydro-2-phenyl-1H-1,2,4-triazole-3-thione (**7**) *by a* Dimroth *Rearrangement.* A soln. of 500 mg (2.43 mmol) of the mixture **1f**/**6** in aq. NaOH (25 ml, 10%) was heated for 5 h at 80°. After this time, TLC (AcOEt/hexane 2:1) revealed only two spots: the isomer **1f**, which had not been involved in the *Dimroth* rearrangement (higher R_f), and the newly formed heterocycle **7** at lower R_f. After adding H_2O and the extraction with CH_2Cl_2, the solvent was removed, and the product was purified by CC (AcOEt/hexane 2:1). Yield 28%. Oil. R_f (AcOEt/hexane 2:1) 0.68. ^{1}H-NMR ($CDCl_3$): 1.38 (*t*, J = 7.6, Me); 2.86 (*q*, J = 7.6, CH_2); 7.36 (*t*, J = 7.4, H–C(4) of Ph); 7.36 (*dd*, J = 7.6, 8.2, H–C(3), H–C(5) of Ph); 7.66 (*t*, J = 8.2, H–C(2), H–C(6) of Ph); 8.44 (*s*, NH). ^{13}C-NMR ($CDCl_3$): 12.4 (Me); 21.8 (CH_2); 119.9, 127.8, 129.7, 141.0 (C(2), C(6); C(4); C(3), C(5); C(1) of Ph), 164.5 (C(5)); 177.2 (C=S).

Viruses and Cells. The origin of the HIV-1(III_B) virus stock has been described in [22]. HIV-1 and HIV-2(ROD) [23] stocks were obtained from the culture supernatant of HIV-1- or HIV-2-infected MT-4 cells, resp. [24]. Simian immunodeficiency virus (SIV(mac251)) was originally isolated by *Daniel et al.* [25] and was obtained from *C. Bruck* (*Smith-Kline-RIT*, Rixensart, Belgium). SIV(mac251) stocks were prepared from the supernatant of SIV-infected MT-4 cells. S0561945 is an HIV-1(III_B) strain, possessing the K103N and Y181C mutations in its RT gene, resulting in resistance towards nonnucleoside RT inhibitors (NNRTIs) [26].

Antiviral Activity and Cytotoxicity Assays. The inhibitory effects of a series of substituted imidazole and 1,2,4-triazole derivatives on HIV-1, HIV-2, and SIV replication were monitored by measuring the viability of MT-4 cells at 5 d after infection. Cytotoxicity of the compounds was determined in parallel by measuring the viability of mock-infected cells on day 5. The number of viable cells was quantified semi-automatically by a tetrazolium-based colorimetric method with 3-(4,5-dimethylthiazolyl-2-yl)-2,5-diphenyltetrazolium (MTT), as described by *Pauwels et al.* [9].

Reverse Transcriptase (RT) Assays. For determination of the 50% inhibitory concentration (IC_{50}) of the test compounds against HIV-1 RT, the RNA-dependent DNA polymerase assay was performed as follows: the reaction mixture (50 µl) contained 50 mM *Tris*·HCl (pH 7.8), 5 mM DTT, 300 µM glutathione, 500 µM EDTA, 150 mM KCl, 5 mM $MgCl_2$, 1.25 µg of bovine serum albumin, a fixed concentration of the labelled substrate [^{3}H]dGTP or [^{3}H]dTTP (*ca.* 6 µM), a fixed concentration of the template/primer poly (rC·oligo-dG) or poly (A·oligo-dT) (0.1 mM; *Amersham Pharmacia Biotech*), 0.06% *Triton X*-100, 5 µl of inhibitor solution (containing various concentrations (10-fold dilutions) of the compounds), and 5 µl of the RT preparations. The reaction mixtures were incubated at 37° for 30 min; then 200 µl of yeast RNA (2 mg/ml) and 1 ml of CCl_2COOH (5% (*v*/*v*)) in sat. phosphate buffer were added. The solns. were kept on ice for at least 15 min; then the acid-insoluble material was filtered over *Whatman GF/C* glass-fiber filters and washed with 5% CCl_3COOH in H_2O and EtOH. The filters were then analyzed for radioactivity in a liquid scintillation counter (*Canberra Packard*, Zellik, Belgium). The IC_{50} value for each test compound was determined as the compound concentration that inhibited HIV RT activity by 50%.

We are grateful to *Cindy Heens*, *Kristien Erven*, *Liesbet De Dier*, and *Lizette van Berckelaer* for their excellent technical assistance, and to *Christiane Callebaut* for dedicated editorial help. We thank Prof. *Jef Rozenski* for the mass spectrometry. *I. Lagoja* is a research associate of the *Rega Foundation*. We thank *ISEP/FORTIS* and the *European Commission* for financial support. Results of this research are subject of a patent application (I. M. Lagoja, C. Pannecouque, A. Van Aerschot, P. Herdewijn, and E. De Clercq, Patent appl. PCT/EPOI/02140, 24.02.2001).

REFERENCES

[1] E. De Clercq, *J. Med. Chem.* **1995**, *38*, 2491.
[2] a) J. M. Coffin, *Science* **1995**, *267*, 483–489; b) A.-M. Vandamme, K. Van Vaerenbergh, E. De Clercq, *Antiviral Chem. Chemother.* **1998**, *9*, 187.
[3] a) E. De Clercq, *Rev. Med. Virol.* **2000**, *10*, 255; b) J. P. Moore, M. Stevenson, *Nat. Rev. Mol. Cell Biol.* **2000**, *1*, 40–49.
[4] R. A. Katz, A.-M. Skalka, *Annu. Rev. Biochem.* **1994**, *63*, 133.
[5] H. Jonckheere, J. Anné, E. De Clercq, *Med. Res. Rev.* **2000**, *20*, 129.
[6] R. Esnouf, J. Ren, C. Ross, Y. Jones, D. Stammers, D. Stuart, *Nat. Struct. Biol.* **1995**, *2*, 303.
[7] T. Fujiwara, A. Sato, M. El-Farrash, S. Miki, K. Abe, Y. Isaka, M. Kodama, Y. Wu, L. B. Chen, H. Harada, H. Sugimoto, M. Hatanaka, Y. Hinuma, *Antimicrob. Agents Chemother.* **1998**, *42*, *1340*.
[8] J. Ren, C. Nichols, L. E. Bird, T. Fujiwara, H. Sugimoto, D. I. Stuart, D. K. Stammers, *J. Biol. Chem.* **2000**, *275*, 14316.
[9] R. Pauwels, J. Balzarini, M. Baba, R. Snoeck, D. Schols, P. Herdewijn, J. Desmyter, E. De Clercq, *J. Virol. Methods* **1988**, *20*, 309.
[10] a) W. Lunkenheimer, G. Jaeger, F. Hoffmeister, Deutsches Patent DT2440378 A1, 1974; b) E. S. H. El Ashry, L. F. Awad, M. Winkler, *J. Chem. Soc., Perkin Trans.*, **2000**, 829, 834.
[11] H. Schildknecht, G. Renner, *Liebigs Ann. Chem.* **1972**, *761*, 189.
[12] D. L. Comins, G. Jianhua, *Tetrahedron. Lett.* **1994**, *35*, 2819.
[13] A. Sitte, R. Wessel, H. Paul, *Monatsh. Chemie* **1975**, *106*, 1291.
[14] M. Pesson, S. Dupin, G. Polmans, *Bull. Soc. Chim. Fr.* **1961**, 1581.
[15] F. Mohamadi, N. G. J. Richards, W. C. Guida, R. Liskamp, M. Liton, C. Caufield, G. Chang, T. Hendrickson, W. C. Still, *J. Comput. Chem.* **1990**, *11*, 440.
[16] S. K. Kearsley, G. M. Smith, *Tetrahedron Comput. Methodol.* **1990**, *3*, 615.
[17] L. Ingber, *Math. Comp. Modelling* **1993**, *18*, 29.
[18] W. D. Cornell, P. Cieplak, C. I. Bayly, I. R. Gould, K. M. Mertz, D. M. Ferguson, D. C. Spellmeyer, T. Fox, J. W. Caldwell, P. A. Kollman, *J. Am. Chem. Soc.* **1995**, *117*, 5179.
[19] M. W. Schmidt, K. K. Baldridge, J. A. Boatz, S. T. Elbert, M. S. Gordon, J. H. Jensen, S. Koseki, N. Matsunaga, K. A. Nguyen, S. Su, T. L. Windus, M. Dupuis, J. A. Montgomery, *J. Comput. Chem.* **1993**, *14*, 1347.
[20] C. I. Bayly, P. Cieplak, W. D. Cornell, P. A. Kollman, *J. Phys. Chem.* **1993**, *97*, 10269.
[21] R. A. Sayle, E. J. Milner-White, *TIBS* **1995**, *20*, 374.
[22] M. Popovic, M. G. Sarngadharan, E. Read, R. C. Gallo, *Science* **1984**, *224*, 497.
[23] F. Barré-Sinoussi, J. C. Chermann, M. T. Nugeyre, S. Chamaret, J. Gruest, C. Dauguet, C. Axler-Blin, F. Vézinet-Brun, C. Rouzioux, W. Rozenbaum, L. Montagnier, *Science* **1983**, *220*, 868.
[24] S. Harada, Y. Koyanagi, N. Yamamoto, *Science* **1985**, *229*, 563.
[25] M. D. Daniel, N. L. Letvin, P. K. Sehgal, G. Hunsmann, D. K. Schmidt, N. W. King, R. C. Desrosiers, *J. Gen. Virol.* **1987**, *68*, 3183.
[26] M. Witvrouw, W. Pluymers, N. Neamati, T. Burke, G. Pais, E. De Clercq, C. Pannecouque, Z. Debyser, Abstracts of 14th International Conference on Antiviral Research, Seattle, Washington, USA; *Antiviral Res.* **2001**, *50*, No. 1: A49 abstract No. 33.

Received May 6, 2002